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Effect of copolymer composition on particle morphology and release behavior *in vitro* using progesterone

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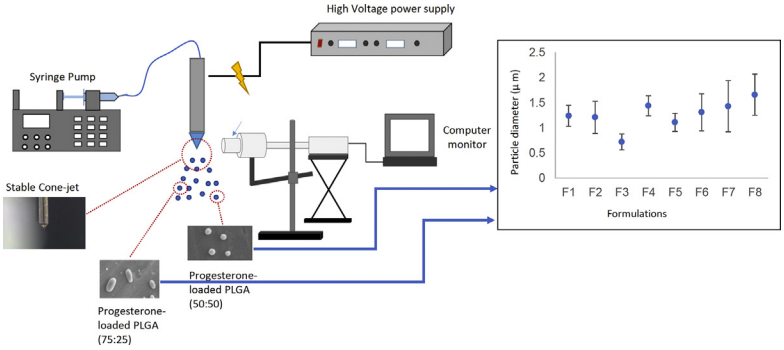
HIGHLIGHTS

- Single step electrospraying was applied with high processing yield to prepare drug delivery systems.
- Solutions of PLGA with different monomer and drug ratios were used for producing progesterone-loaded microparticles.
- Encapsulation efficiency of 80–90% was achieved in selected formulations.
- The release kinetics were studied *in vitro* and the progesterone release rate was controlled.
- The dissolution rate of hydrophobic progesterone in the prepared formulations was significantly improved.

GRAPHICAL ABSTRACT



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ABSTRACT

This study was aimed at improving dissolution rate and sustained release of progesterone by varying copolymer composition and polymer: drug ratio of PLGA. Drug-loaded particles were prepared using electrohydrodynamic atomization. The effects of polymer: drug ratio and copolymer composition on particle properties and *in vitro* drug-release profile were investigated. The physical form of the generated particles was determined *via* X-ray powder diffraction (XRPD) and Fourier transform infrared spectroscopy (FTIR). Drug release *in vitro* was found to be dependent on copolymer composition, where the release rate increased with decreased lactide content of PLGA. Particles produced with solutions of copolymer (75:25) had elongated shapes. In general, the obtained results indicated that the prepared microparticles were ideal carriers for oral administration of progesterone offering great potential to improve the dissolution rate of drugs that suffer from low aqueous solubility.

1. Introduction

In the development of oral formulations, the aqueous solubility of the drug plays a key role in the extent of drug absorption [1]. For any therapeutics to be successfully absorbed in the body, it needs to be present in the form of an aqueous solution at the desired site of action [1,2]. The solubility is the limiting factor for a drug to attain the desired concentration in systemic circulation in order to achieve pharmacological response for an orally administered drug [3]. However, many drug molecules under development are poorly soluble: that critically limits their absorption, resulting in poor bioavailability and pharmacokinetics *in vivo* [2,3].

Progesterone is an endogenous steroidal hormone that is involved in all aspects of reproduction and used to control reproductive function. It

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is being used for hormone replacement therapy (HRT) in menopausal women, who failed to ovulate and stop producing progesterone from their ovaries [4], this would drop down the hormone levels in the body, hence results in various effects such as hot flashes and night sweats. In addition, recent studies have reported the applicability of progesterone in preventing preterm birth. This is defined by World Health Organization as birth before 37 weeks of pregnancy are completed [5], which is the largest cause of neonatal morbidity and mortality in infants [6,7]. Progesterone has an essential role in maintaining pregnancy [8,9], and it is involved in establishing uterine quiescence [10]. The various administration routes of progesterone include oral, parental (intramuscular and subcutaneous) and topical (as a cream of vaginal gel), of which peroral delivery is the most preferred route of administration. However, progesterone suffers from a short half-life and low water solubility; these lead to inconsistent bioavailability and high first pass metabolism [11]. Biodegradable polymers are promising candidates for oral drug delivery with their ability to shield the drug from external harsh chemical and enzymatic degradation of the gastrointestinal tract (GIT) as well as bypass the first pass metabolism through their unique uptake mechanism [12,13]. A previous study reveals that the dissolution rate of poorly water-soluble drug can be enhanced by incorporating it into a polymeric matrix thereby inducing its amorphous state [14], again making this a preferable oral delivery technique. In addition to this, long term sustained drug delivery systems can also be obtained using biodegradable particles, in which progesterone can be administered *via* depot injection [15] and released for a prolonged period of time. In a depot injection, the medication is deposited in a localized mass *via* intramuscularly, subcutaneously or intradermal injection. This offers advantages over long-term daily injection which can compromise patient compliance.

Biodegradable microparticles in the field of controlled drug release, where the drug is continuously released from the particles within a period of time, have generated immense interest. They are able to deliver a wide range of drugs through various administration routes and enhance drug bioavailability and protect the drug from degradation [16]. The drug release behavior can be controlled by altering polymer compositions including molecular weight, monomers ratio and the nature of terminal groups for copolymers [17]. It has been reported that other than the physicochemical properties of polymers, factors such as particle size [18,19], morphology and porosity [20], as well as drug loading [21] also play important roles in deciding the drug release kinetics. Appropriate selection of the biodegradable polymeric materials is essential to develop a successful particulate delivery system. Among the variety of materials available to fabricate these solid particles, copolymers have received much attention in recent years as different physicochemical properties can be achieved by altering their polymer composition.

Poly (D, L lactic *co* glycolic acid) (PLGA) is one of the most frequently used biodegradable polymers in drug delivery applications [22,23]. PLGA is approved by the FDA as a drug delivery vehicle and it preferred for this purpose because of its favorable biodegradability, biocompatibility and tunable physicochemical properties [24,25]. The copolymers with various molecular weights and copolymer compositions are commercially available. The ability to alter lactide: glycolide ratio, molecular weight and the nature of terminal groups enables precise control over drug release profile [25]. In general, therapeutics can be orally released in a controlled manner *via* transport through the polymer or be triggered in response to environmental stimuli or released during polymer dissolution [26]. In addition, the bioavailability of hydrophobic drugs can be improved due to the hydrophilic property of PLGA, thus enhancing oral bioavailability [27,28].

Polymeric micro- and nanoparticles can be administered as oral, injectable and inhalable drug delivery systems, and can be fabricated using various methods including solvent evaporation [29], emulsion [30,31], spray-drying [32,33], porous glass membrane emulsification [34] and coacervation [35]. However, most of these conventional approaches suffer from some limitations such as broad particle-size distribution, low drug encapsulation efficiency [36], particle agglomeration and difficulties for incorporation of hydrophilic drugs [30,37]. Moreover, non-degradable surfactants are employed in some of the approaches which might cause unwanted effects in pharmaceutical applications. In addition, for highly sensitive and active pharmaceutical ingredients, inactivation or degradation is possible owing to high temperature, organic solvent exposure and high shear stress during the particle fabrication process [31,38]. Electrohydrodynamic atomization (EHDA), also known as the electrospray technique, is believed to overcome the drawbacks associated with conventional techniques and has been deliberately chosen as a method for the fabrication of bioactive molecule loaded micro and nanostructured materials in the application of drug delivery [39–41]. In this method, drug molecules are firstly incorporated into the polymer solution and infused through a capillary nozzle. A high voltage is applied to atomize the solution and the electric charge generated on the droplet, competing with the surface tension and resulting in near-monodisperse microdroplets in the cone-jet mode and further solidify to form microspheres in a single-step process. EHDA also has the potential to avoid particle aggregation due to self-dispersing of the droplets owing to Coulombic repulsion [42]. Moreover, electrospraying has the advantage of high drug encapsulation efficiency [43] and can enable encapsulation of both hydrophobic and hydrophilic drugs [28,39].

In this work, electrospraying was utilized to produce progesterone loaded PLGA particles. Many studies have investigated the formulations of PLGA particles for controlled release of different pharmaceuticals [16,18,25,43,44] and encapsulated hydrophobic drugs within different polymer matrices [11,27,28]. Progesterone-loaded PLGA microparticles have been prepared by several researchers by use of different techniques, including microfluidic [26], hydrogel template [45] and solvent evaporation-based methods [46]. However, there is a lack of systematic reports on applying single step electrospraying in producing progesterone-loaded particles, specifically using PLGA copolymer with different monomers ratios and concentrations which can provide different drug release profile in order to meet various requirements of drug delivery. Given the hydrophobicity of progesterone and that oral delivery is the most preferred route of administration, a particular challenge and objective was to enhance its dissolution by incorporation of the drug into the PLGA matrix. The overall objective of this study was to carry out a systematic investigation on the effects of the electrospraying technique, polymer concentration and use of two different copolymer compositions (PLA/PGA ratio of 50:50 and 75:25) on the resulting particle properties, and release behavior (*in vitro*) using progesterone.

2. Material and methods

2.1. Materials

PLGAs with different copolymer ratios (1. PURASORB PDLG 5002A, 50:50 DL-lactide/glycolide copolymer, molecular weight of 17,000 Da, inherent viscosity 0.2 dl g^{-1}; 2. PURASORB PDLG 7502A, 75:25 DL-lactide/glycolide copolymer, molecular weight of 17,000 Da, inherent viscosity 0.2 dl g^{-1}) was obtained from Corbion (Amsterdam, The Netherlands). Progesterone (molecular weight of 314.46 g mol^{-1}) and *N*, *N* Dimethylacetamide (DMAc, anhydrous, 99.8%) were obtained from Sigma-Aldrich (Poole, UK).

2.2. Particle preparation

Progesterone-loaded PLGA particles were produced using electrospraying (Fig. 1). PLGA solutions with different concentrations (2 and 4 wt%) and copolymer formulations (50:50 and 75:25) were prepared by dissolving the polymer in DMAc at ambient temperature (20 °C). Progesterone (2 mg/ml) was added to polymer solution at each concentration to make polymer/drug ratios of 20:1 and 10:1.

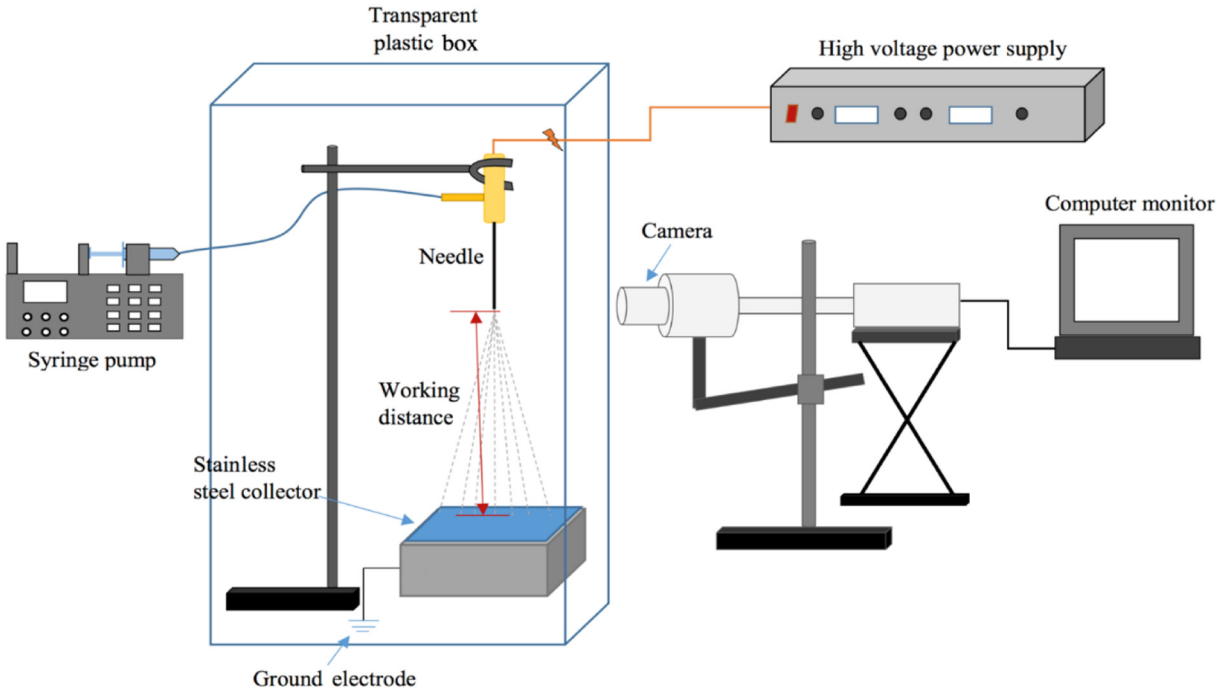


Fig. 1. Electrohydrodynamic atomization setup.

PLGA solutions with no drug (2 and 4 wt%; 50:50 and 75:25) were prepared as a control study.

The polymer solutions were loaded into a 10 ml plastic syringe and fed into a stainless-steel needle with internal diameter of 0.84 mm at a constant flow rate implemented by a syringe pump (Harvard PHD 4400, Edenbridge, UK). A high positive electrical potential varying from 12.0 to 16.0 kV relative to the ground was supplied using a high voltage power supply (Glassman Europe Ltd., UK). Once the droplets were ejected from the stable cone-jet, particles were collected at a working distance of 200 mm below the needle exit, either on a glass microscope slide, or a grounded collector. The cone-jet formed at the tip of the needle during particle production was monitored using a Leica DMS300 camera. All experiments were conducted at ambient temperature (20 °C) and relative humidity of 40–55%. Each experiment was repeated 3 times.

2.3. Characterization of solutions

The density (ρ) was measured using a standard density bottle (DIN ISO 3507-Gray-Lussac). Surface tensions (γ) of all the solutions were determined using Wilhelmy's plate method using a Kruss tensionmeter (Tensiometer K9, Hamburg, Germany). The viscosity (μ) was estimated using a U-tube viscometer (VWR, size E). The electrical conductivity (κ) was measured using a conductivity probe (Jenway 3540 pH/conductivity meter). The characterization of all solutions was undertaken at ambient temperature. The mean value and standard deviation of ten readings is reported. In order to investigate how PLGA copolymer ratio and concentration influence the size, morphology and eventually the release profile of the particles, four solutions with various PLGA concentrations (2 and 4 wt%), different copolymer ratios (50:50 and 75:25) were prepared. Progesterone was added to each solution to make four more solutions incorporating the drug. The measured values for these parameters are summarized in Table 1.

2.4. Particle size and zeta potential

Particles were collected on glass slides and initially examined using an optical microscope (Nikon Eclipse ME 600) fitted with a camera (Micropublisher 3.3 RTV, 3.3 megapixel CCD Color-Bayer Mosaic, Real Time Viewing camera; Media Cybernetics, Marlow, UK). Particles were further observed using a field emission scanning electron microscope (SEM; Hitachi) for particle size and morphology analysis. The micrographs were taken from different areas for each sample and 300 particles were randomly chosen to measure both width and length using ImageJ (Brocken Symmetry Software). To measure the zeta potential of particles, a dilute suspension of particles was prepared in distilled water. The zeta potential was estimated with ZetaPlus™ zeta potential analyzer (Brookhaven Instruments, Holtsville, NY) at room temperature on the basis of electrophoretic mobility under an electric field. For each formulation the mean value of 5 measurements were taken.

Table 1
Physicochemical characteristics of the solutions used for particle formation. In the case of viscosity, electrical conductivity and density the error was negligible.

Formulation	Polymer concentration and drug loading (with respect to polymer)	Surface tension (mN m^{-1})	Viscosity (mPa s)	Electrical conductivity (μS cm^{-1})	Density (*kg* l^{-1})
F1	2 wt% (50/50) + 10 wt% drug	30.4 ± 0.7	1.2	1.1	9.4
F2	2 wt% (75/25) + 10 wt% drug	32.1 ± 0.6	1.1	0.8	9.3
F3	4 wt% (50/50) + 5 wt% drug	28.9 ± 0.8	1.2	1.0	9.4
F4	4 wt% (75/25) + 5 wt% drug	28.4 ± 1.1	1.1	0.9	9.4
F5	2 wt% (50/50)	31.3 ± 0.6	1.4	1.1	9.4
F6	2 wt% (75/25)	28.2 ± 0.4	1.2	0.9	9.3
F7	4 wt% (50/50)	33.7 ± 0.8	1.4	1.1	9.4
F8	4 wt% (75/25)	34.5 ± 0.8	1.3	1.0	9.4

2.5. Production yield and drug encapsulation efficiency

The production yield of the processing technique was calculated as the ratio of the mass of dried particles collected (M_c) to the theoretical mass of particles sprayed during the collection time (M_i).

$$production\ yield\ (\%) = \left(\frac{M_c}{M_i}\right) \times 100\% \quad (1)$$

$$M_i = polymer\ concentration \times flow\ rate \times collection\ time \quad (2)$$

Drug encapsulation efficiency was determined by measuring the amount of drug in the collected particles. Samples with 10 mg of progesterone-loaded particles were weighed, dissolved with DMAc (10 ml) and mechanically stirred for 400 s. The solution was then diluted 1:10 in DMAc: PBS (10: 90 v/v) and filtered through 0.22 μm filter. The drug content was analyzed using UV–visible spectrophotometer at 224 nm detection wave length. The concentration was then calculated using a calibration equation.

$$\text{Encapsulation Efficiency} = \frac{mass\ of\ actual\ drug\ loaded\ in\ particles}{mass\ of\ drug\ used\ in\ particle\ fabrication} \times 100\% \quad (3)$$

2.6. X-ray powder diffraction

XRPD patterns of samples were analyzed. The X-rays are generated by a cathode ray tube filtered to produce monochromatic radiation directed towards the sample. Cu Kα radiation was used with wavelength 1.5418 Å and graphite monochromatic filtering wave at a tube voltage of 40 mV and tube current of 15 mA. The interaction of the incident rays with the sample produces constructive interference (and diffracted rays). The diffracted intensity was recorded in the 2θ angle range from 5 to 40° at a scanning speed of 0.02°/min.

2.7. Fourier transform infrared spectroscopy

The infrared spectra of two PLGA formulations, progesterone and drug-loaded particles, were recorded using a Fourier Transform Infrared spectrophotometer. A small quantity of each sample was placed onto the sample holder and scanned in the frequency range of 400–4000 cm^{-1}.

2.8. In vitro drug release of progesterone

To investigate the release profile of progesterone-loaded particles, *in vitro* dissolution studies were conducted in phosphate-buffered saline (PBS) with pH 7.4. One compressed phosphate-buffer saline tablet (PBS, yields 0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride, pH 7.4 at 25 °C, Sigma-Aldrich, UK) was dissolved in 200 ml of deionized water. 22 mg of progesterone-loaded particles were encapsulated in a dissolvable polymer capsule. The solubility of 2 mg of pure progesterone was investigated as a control group under the same release conditions in order to investigate how solubility of poorly water-soluble progesterone can be enhanced after electrospraying in the PLGA matrix. The polymer capsule was then put in a stainless-steel sinker basket. The sinker basket was subsequently submerged into 100 ml PBS and the temperature of the solution was kept constant at 37 °C. At predetermined time intervals, samples of 3 ml were withdrawn from each solution and immediately replaced with fresh PBS already stored at 37 °C. Dissolution studies were also conducted on pure progesterone under the same protocol as the control group. The samples were filtered through a membrane (0.22 μm) to filter any impurities and avoid any interference in absorbance. Three replicates of each sample were analyzed for drug concentration using UV–visible spectrophotometer at 224 nm to calculate the drug percentage. A calibration equation to calculate the progesterone concentrations of unknown samples was produced by measuring the absorbance of different concentrations of progesterone from 1 to 20 μg/ml. The release studies were conducted over 7 days and repeated three times for each set of PLGA particles and the results of cumulative amount released was plotted as a function of time.

2.9. Analytical model of drug release

The release of drugs from PLGA particles is known to be a complicated process, involving both diffusion of the drug out of the particles and inward diffusion of water leading to modification of the PLGA. Detailed mathematical models of these processes have been set up [47,48], and although they can be applied quite generally they have most often been used with perfect sink boundary conditions, that is, the drug concentration at the surface has been set to zero. In the experiments reported here the drug solubility is low, and the solution in the container is saturated after about 120 min and remains approximately constant. In the saturation regime the rate of release of the drug will be determined by the intervals between sampling the solution and replenishing it rather than by the rate at which the drug can escape from the particles.

During the early stages of release, it is reasonable to assume that simple diffusion of the drug through the PLGA matrix is the rate-controlling process. Under those circumstances a convenient approximation to the amount released after time t is given by the expression [49]:

$$Q(t) = Q_{max}\ tanh\left(\frac{\beta(Dt)^{\frac{1}{2}}}{a}\right) \quad (4)$$

where Q_{max} is the amount released at saturation, D is the diffusion coefficient of the drug in the PLGA, a is the radius of the particles, and β is a fitting parameter, equal to 3.345. Although Eq. (4) was derived for spherical particles, it will also be a good approximation for the elongated particles found in some of the experiments, as a similar expression has been found to be a good descriptor of release from long tubes, where β is 2.257.

3. Results and discussion

3.1. Fabrication of microparticles

Four different formulations of drug-loaded particles and PLGA particles were successfully prepared using electrospraying under different operating conditions (Table 2). The model drug, progesterone, was encapsulated in PLGA polymers by co-dissolving in DMAc before electropraying into fine droplets. The effects of PLGA concentration and copolymer composition were evaluated by particle characterization and drug-release behavior in this study. Other important parameters such as applied voltage and solution flow rate were optimized to achieve a stable cone-jet and uniform size distribution.

3.2. Characterization of microparticles

3.2.1. Particle size and geometry

3.2.1.1. Copolymer ratio and polymer/drug ratio. Particles were produced both with and without progesterone to investigate the effects of polymer/drug ratio and copolymer formulation of PLGA on particle size and morphology. Initially, drug free particles were produced as the control system using PLGA with two copolymer ratios (50:50 and 75:25), and for each copolymer formulation, particles were prepared using PLGA concentrations of 2 and 4 wt% (F5, F6, F7 and F8, respectively). Drug free particles were generated at constant flow rate of 3 μl/min

Table 2
Operating conditions tested and corresponding characterization. F2, F4, F6, and F8 particles are oblong and have two dimensions.

Formulations	Processing Parameters	Average Particle Dimensions (μm)	Yield (%)	Encapsulation Efficiency (%)	Zeta Potential (mV)
F1	2 μl/min 12.6 kV	1.24 ± 0.21	81 ± 3	88 ±2	−44.60 ± 4.19
F2	2 μl/min 13.8 kV	1.21 ± 0.32 0.66 ± 0.09	83 ± 2	83 ±3	−43.01 ± 5.65
F3	2 μl/min 15.1 kV	0.72 ± 0.16	83 ± 2	83 ±3	−47.78 ± 3.85
F4	2 μl/min 15.7 kV	1.44 ± 0.2 0.63 ± 0.11	83 ± 2	83 ±3	−44.82 ± 3.49
F5	3 μl/min 15.3 kV	1.1 ± 0.18	85 ± 2	–	−34.40 ± 3.87
F6	3 μl/min 15.8 kV	1.31 ± 0.37 0.64 ± 0.12	85 ± 1	–	−35.87 ± 3.87
F7	3 μl/min 13.8 kV	1.43 ± 0.51	80 ± 1	–	−38.39 ± 4.21
F8	3 μl/min 14 kV	1.66 ± 0.41 1.2 ± 0.33	84 ± 2	–	−39.32 ± 3.34

and the voltage required to form a stable cone jet was between 13.0 and 16.0 kV. The particles size and morphology were studied using SEM, and the representative SEM images of each formulation are shown in Fig. 2. In general, all particles were produced with a smooth outer surface. The results showed that both concentration and copolymer ratio of the polymer influenced the diameter of progesterone-free and progesterone-loaded particles. Larger particles were produced with higher concentrations of PLGA while the outer surface of the particles stayed smooth, which can be attributed to the higher viscosity of the 4 wt% polymer solutions (values are shown in Table 1). The copolymer ratio and addition of drug were found to have no significant influence on particle size.

SEM images (Fig. 2) showed that the solution from PLGA copolymer ratio of 50:50 for both polymer concentrations resulted in formation of spherical particles, while solutions from PLA: PGA copolymer ratio of 75:25 produced elongated or irregular particles.

3.2.1.2. Flow rate and voltage. The size of the electrosprayed particles can be tailored by changes in voltage and flow rate. According to Hartman and colleagues [50], reducing the polymer solution flow rate reduces particle size, as shown in Eqs. (5), (6), and (7).

$$d = c\left(\frac{\rho\varepsilon_0 Q^4}{I^2}\right)^{1/6} \tag{5}$$

$$I \propto (\gamma\kappa Q)^{1/2} \tag{6}$$

$$d \propto Q^{1/2} \tag{7}$$

In Eqs. (5), (6), and (7) [50] d is the particle diameter (m), c is a constant, Q the liquid flow rate ($m^3\ s^{-1}$), ρ the liquid density, I the current, ε_0 the permittivity of vacuum, γ the liquid surface tension in ambient air, and κ the liquid electrical conductivity.

In order to investigate the effect of copolymer formulation on progesterone release profile, particles obtained from the same polymer concentration are required to have similar dimensions. Thus, flow rate was kept constant at 2 μl/min in order to achieve comparable particle size distribution. The diameter of the particles obtained from all formulations are in the range 0.72 and 1.66 μm and presented in Table 2, however note well that some are oblong in shape.

3.2.2. Zeta potential

Zeta potential is an important characteristic of polymeric particles, it is a measurement of surface charge of the particles in a suspension. In a sense, zeta potential can influence particle stability, as the absolute value of zeta potential increases the amount of charge on the particle surface. This results in larger electrostatic repulsive interaction between particles which prevents the aggregation of the particles and leads to the formation of more stable particles. A physically stable particle suspension should have a minimum zeta potential of ±30 mV [51]. The zeta potentials of all the formulations ranged between about −30 mV and −50 mV. It showed slight increases in negativity with an increase in polymer concentration and addition of drug. PLGA composition was found to have no significant influence on zeta potential.

3.2.3. X-ray diffraction

The physical form of the drug, polymer and drug-loaded particles were tested using XRPD and the patterns are shown in Fig. 3. The diffractogram of progesterone shows strong characteristic peaks at 2θ values of 10.48, 12.64 and 16.9 degrees, which indicate that it is crystalline in its pure form. Both PLGA diffractograms have the characteristics of amorphous material. Electrosprayed particles in all formulations were tested, and no crystalline peaks were observed. A possible explanation for this is that progesterone is molecularly dispersed within the PLGA polymeric matrix and almost complete amorphization was achieved.

3.2.4. Fourier transform infrared spectroscopy

FTIR spectra were used to detect whether there was any interaction between PLGA and progesterone during the electrospray process. FTIR spectra of progesterone, PLGA polymers (75:25 and 50:50) and progesterone-loaded particles with different polymer/drug ratio are shown in Fig. 4. The pure progesterone sample exhibited characteristic peaks including bound OH (2800–3000 cm^{-1}) and C═O stretching (1600–1750 cm^{-1}). PLGA polymers showed vibration C═O stretching at 1752 cm^{-1} and C—O stretching at 1000–1250 cm^{-1}. Additionally, weak peaks for C═O stretching were observed between 1600 and 1750 cm^{-1} in four different progesterone-loaded formulations, suggesting the presence of intact progesterone. Thereby, the FTIR results indicate that there were no chemical interactions between polymer and progesterone.

3.3. In-vitro drug release study

Copolymer ratio is one of the key factors affecting the behavior of drug release. By manipulating the copolymer ratio, the release kinetics of the drug can be controlled. Fig. 5 shows cumulative percentages of released progesterone against time plots of electrosprayed microparticles from four formulations. Narrow error bars indicate good reproducibility. Progesterone release from all four formulations showed a biphasic release pattern. Phase I in this biphasic release profile is usually described as initial burst release, which can be due to three reasons: first, drugs that are freely and weakly bound on the particle surface, second, drug release from the pores and cracks of particles with various morphology

[52] and third, drug diffuses out from the polymeric matrix. Later in the modelling analysis, phase I can also be described as diffusion dominated release with a reasonable value for diffusion constant. Phase II is predominately governed by drug diffusion, either through water-filled pores or polymer. The release study showed increase in PLA:PGA ratio from 50:50 to 75:25 decreased the rate of progesterone release. Drug-

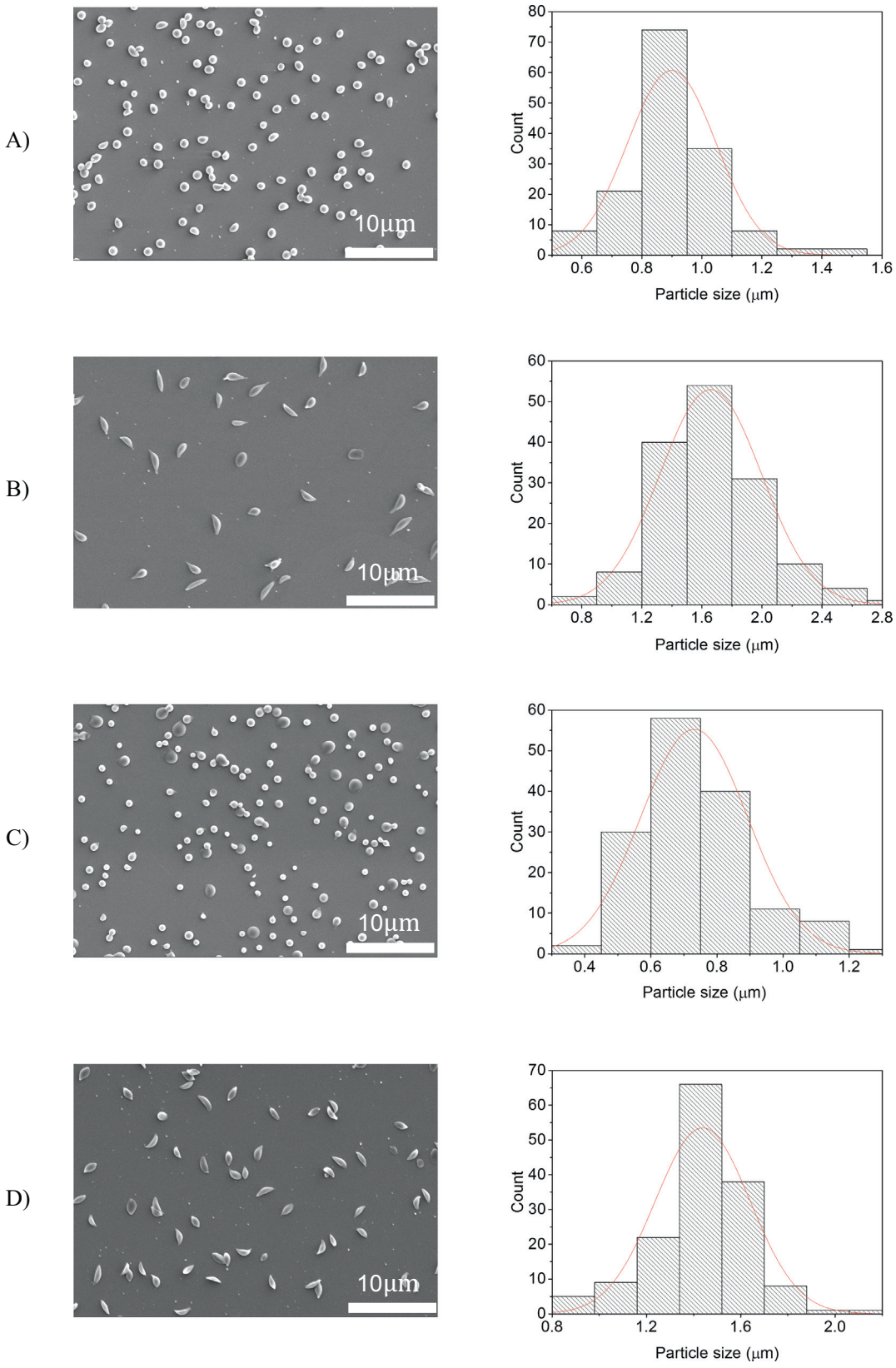
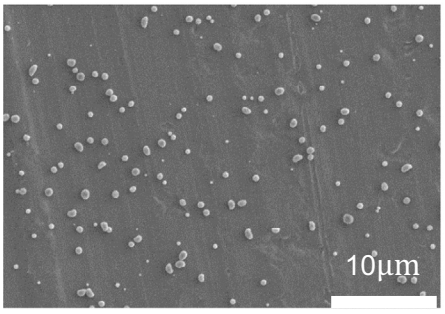
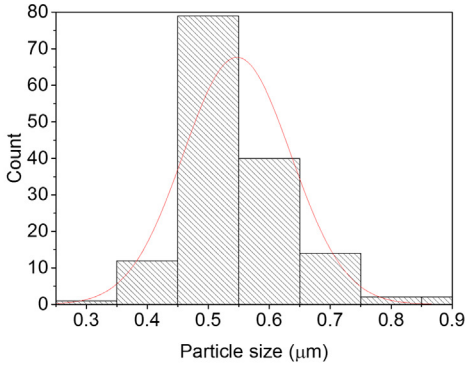


Fig. 2. SEM images and size distribution of drug-loaded particles produced with constant progesterone concentration of 2 mg/ml (a) F1, (b) F2, (c) F3, (d) F4 and drug-free particles e, f, g and h (F4, F6, F7 and F8, respectively).

E)





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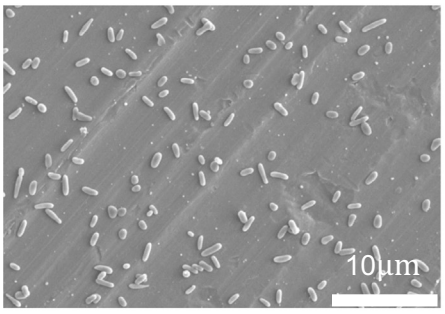

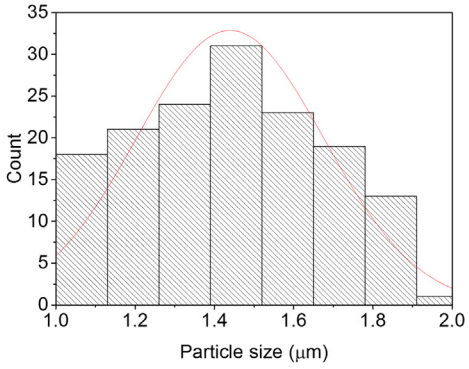

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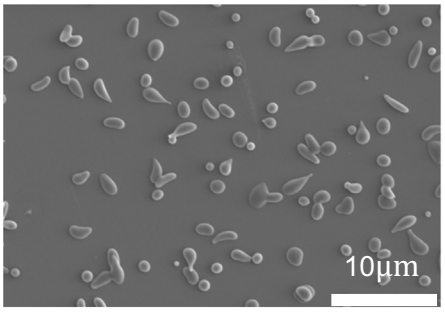
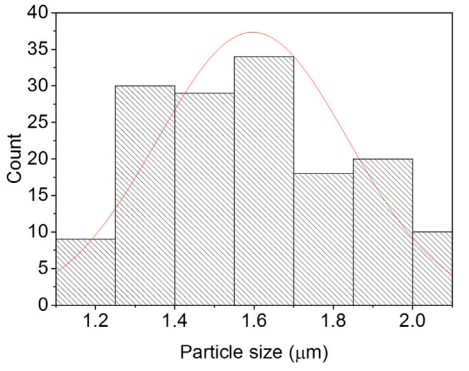
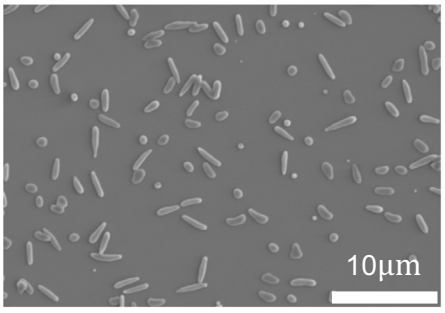




H)



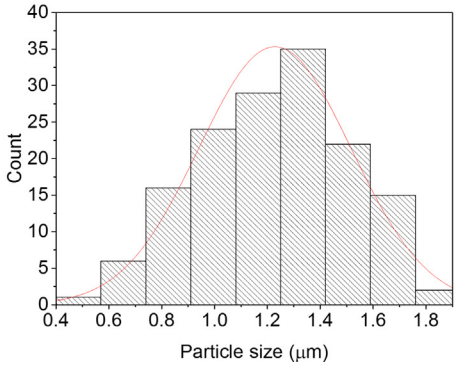


Fig. 2 (*continued*).

loaded PLGA particles of lower PLA:PGA ratio for both polymer concentrations (50:50 2 and 4 wt%) cumulatively released 862 and 792 μg in 2 h, respectively. On the other hand, higher copolymer ratio (75:25 2 and 4 wt%) PLGA particles showed only 741 and 654 μg cumulative release in 2 h, respectively. For diffusion-controlled drug release, water-uptake and swelling ability of polymer will potentially affect the

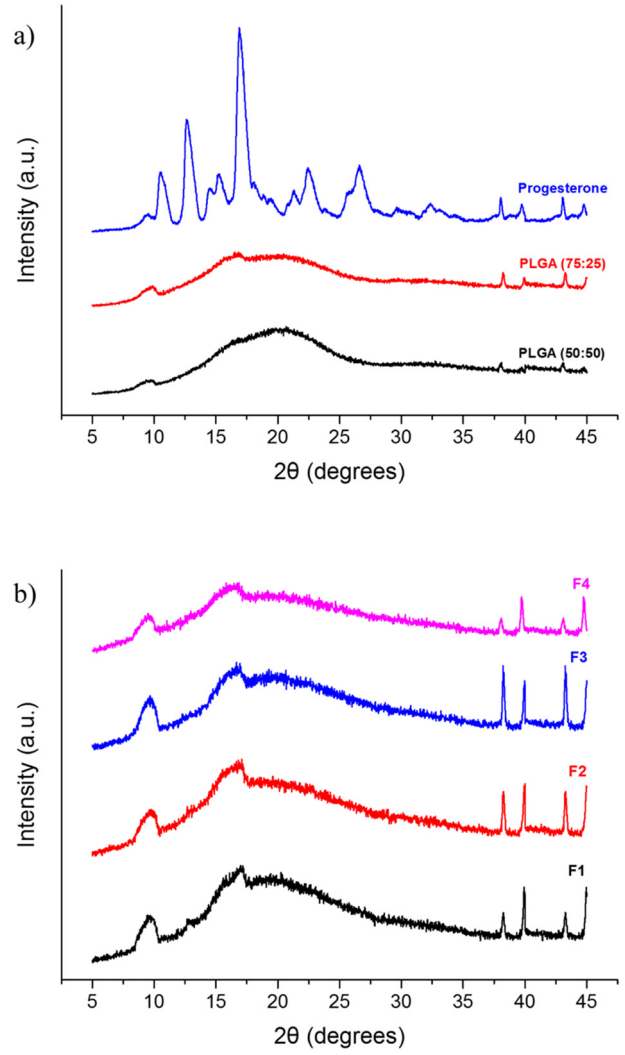


Fig. 3. XRPD patterns of a) progesterone, PLGA (75:25) and PLGA (50:50), b) progesterone-loaded particle formulations F1, F2, F3 and F4.

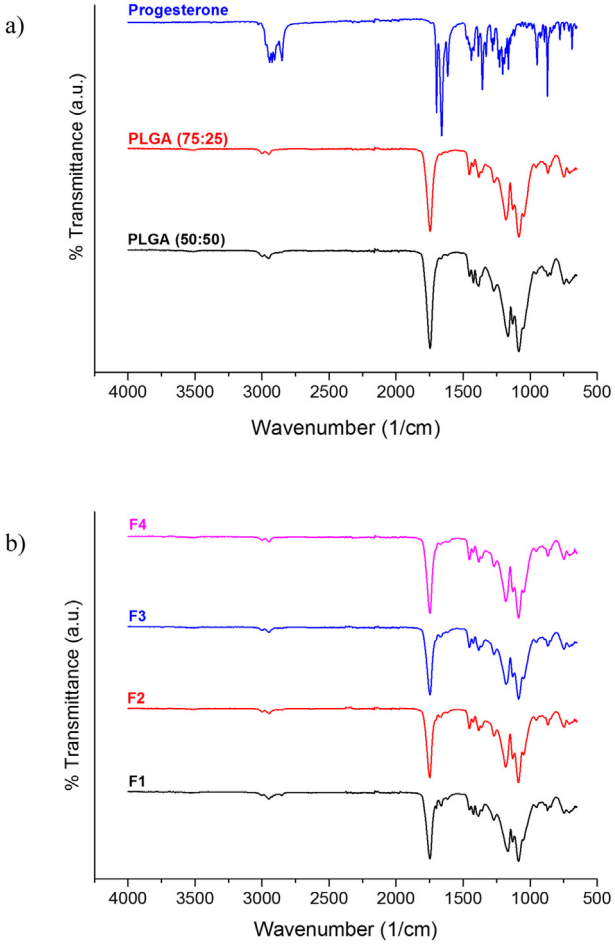


Fig. 4. FTIR spectra of a) progesterone, PLGAs with different copolymer ratios (75:25 and 50:50) and b) electroprayed progesterone-loaded particles of four formulations.

diffusion rates within the polymer matrix. Increase in release rate on decreasing PLA:PGA ratio can be attributed to subsequent increase in hydrophilicity. Water-uptake and polymer hydration occurs immediately upon immersion in water or administration *in vivo* [53]. Lower PLA:PGA ratio (PLGA 50:50 *vs.* 75:25) is considered to be more hydrophilic and thus water absorption rate might increase to a greater extent [54]. This action has been found to be a pore-forming process and leads to an increase in drug diffusion [55,56]. Although, these pores are not big enough for drug transport in the early stage of drug release, a porous network will be established as more and larger pores formed [55,56]. For small hydrophobic drugs, transport through the polymer phase may occur [54,57]. Progesterone is a small hydrophobic drug with average molecular weight of 314.46 g mol^{-1} and low water solubility of 8.81 mg l^{-1} (25 °C) [58]. Since drug must enter the water phase before it can be released, the more hydrophilic the polymer, the faster its water absorption. Hence, PLGA particles with less lactide content, which is therefore more hydrophilic, would provide a better environment for drug to be released [52]. In case of PLGA particles of higher polymer concentration, about 70% of progesterone was released after 2 h, which is before the onset of polymer dissolution. Some of the drug could have been released due to being loosely attached to the surface, but it is unlikely that only few of the drug molecules would have been properly encapsulated in particles. Therefore, it is logical to assume that the release of progesterone was dominated by diffusion through the polymer.

In case of low drug loading (4 wt% PLGA +5 wt% progesterone), the total amount of drug released after 2 h was less (792 μg) compared to 2 wt% PLGA drug-loaded particles (862 μg). For the other copolymer composition (75:25) also, particles with relatively higher polymer concentration showed less cumulative drug release (654 μg) as compared to the lower polymer concentration (741 μg). Besides, an increase in the surface area: volume ratio results in higher release rate of hydrophobic drugs [18,59]. It was noticed that, with the increase in polymer

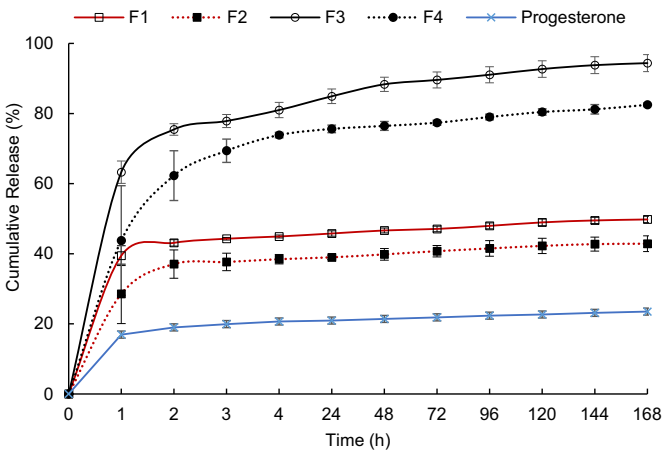


Fig. 5. Release profile of progesterone from all formulations and progesterone dissolution on its own.

concentration, particle size increases resulting in decreased surface area: volume ratio. Therefore, less buffer penetration and hence reduced drug release rate occurs. Also, the increase in particle size may also result in increased drug diffusion distance, which may be another contributing factor. It was noticed that, the main difference was on the initial burst release. The release profile after 48 h in the same copolymer composition seems to have similar patterns. This indicates that after 48 h, PLGA copolymer ratio is playing a dominating role.

It is observed that progesterone is not completely released from most of the formulations during the 168 h of measurement, where only 43, 50, 83 and 94% of progesterone was released from F1, F2, F3 and F4, respectively, indicating that there was still drug entrapped within the polymer network. PLGA erosion typically does not occur within 7 days and this explains the rest of drug within the polymer matrix. As shown in Fig. 5 different progesterone release profiles have been achieved using different PLGA copolymer ratios and concentrations. Administration of progesterone by the oral delivery offers high compliance for patient and is the most preferred route [60]. However, progesterone suffers from low bioavailability and destruction in the digestive system which take place faster than its absorption [61]. As observed, unlike encapsulated progesterone, pure progesterone molecules suffer from extremely low water solubility. As shown in Fig. 5, only 380 μg (24%) progesterone has been dissolved, which is less than half of that from all the other formulations. The dramatic increase in dissolution rate for progesterone loaded PLGA formulations was partially explained by the physical form change of progesterone (Fig. 3) after being electrosprayed with PLGA into particles, which indicates that progesterone is molecularly dispersed within the polymer matrix. As observed, about 85 and 76% of progesterone has been released from formulation F3 and F4 in 24 h, respectively, which can be desirable when rapid treatment is required and make it a preferable oral delivery system. A woman who has her ovaries surgically removed will experience a sudden drop in progesterone level. Such loss of hormone can lead to health problems unless adequate hormone replacement is provided. In such a scenario, rapid oral delivery of progesterone is preferred.

In formulations F1 and F2 only 43 and 37% of progesterone was released after 2 h, respectively, and about 50% was released after 7 days of measurement. This release profile of progesterone is beneficial for sustained release such as by depot injection. As mentioned previously, progesterone has a role in maintaining pregnancy and preventing preterm birth in women considered to be at high risk [6]. To date, the most common routes of administration of progesterone are intramuscular injection and vaginal suppository [62]. In reference [62] the suppository was made and characterized by a new process [63–65]. Progesterone delivered by regular injection may lead to inflammation at the injection site, resulting pain, redness and sterile abscess. Yet, the suppository material for vaginal delivery also has its limitations

Table 3
Diffusion coefficients D for progesterone in PLGA particles, deduced from fitting the early stage release to a simple diffusion model.

Formulation	Size (μm)	D ($m^2 s^{-1}$)
F1	1.24 ± 0.21	$(1.4 \pm 0.4) \times 10^{-17}$
F2	1.21 ± 0.32	$(1.5 \pm 0.4) \times 10^{-18}$
	0.66 ± 0.09	
F3	0.72 ± 0.16	$(6.8 \pm 0.9) \times 10^{-18}$
F4	1.44 ± 0.2	$(1.0 \pm 0.3) \times 10^{-18}$
	0.63 ± 0.11	

such as escaping from the vagina causing inconvenience and uncertainty as to the drug dose absorbed. For women considered to be at high risk of extremely preterm (<28 weeks) and very preterm (28–32 weeks) delivery, long-term administration of progesterone is required in order to maintain its level. Through depot injection [15], the drug is slowly released from the deposits in a localized mass. We believe that such an approach holds several potential benefits including that it ensures sustained release of progesterone at low doses over prolonged periods and prevention of injection site problems by reducing the frequency of dosing. A depot injection is favorable for patients who have difficulty swallowing medication or remembering to take medication regularly.

In general, our data indicates that the electrosprayed polymeric particles are beneficial for formulation of poorly water-soluble drugs for oral administration since they have great potential to enhance the dissolution rate of the drug. The electrospraying process effects the poorly aqueous-soluble molecules at the particulate level through nanonization and amorphization [66]. Under the impact of electrical forces, drugs are dispersed into small droplets ranging from micro- to nanosize, which allows solvent to evaporate easily and, as a result, immediate solidification takes place. By virtue of rapid solvent evaporation amorphous state of drug in the system is generated which attributed to randomly "frozen" drug molecules in the solid polymer matrix forming solid dispersion. Not only a predictable and controlled drug release can be designed by manipulating the polymer concentration and copolymer ratio but also a protection of encapsulated active pharmaceutical ingredients from degradation and loss of bioactivity.

3.4. Mathematical modelling

We have fitted $Q(t)$ in Eq. (4) to the experimental release rates up to 105 min, and the results are shown in Fig. 6. Using the particle sizes in Table 2 we can extract diffusion coefficients for progesterone in PLGA, and the results are shown in Table 3. It appears that the diffusion coefficient in the 50:50 formulation is about ten times greater than in the 75:25 formulation. As a check of accuracy of these values, we have

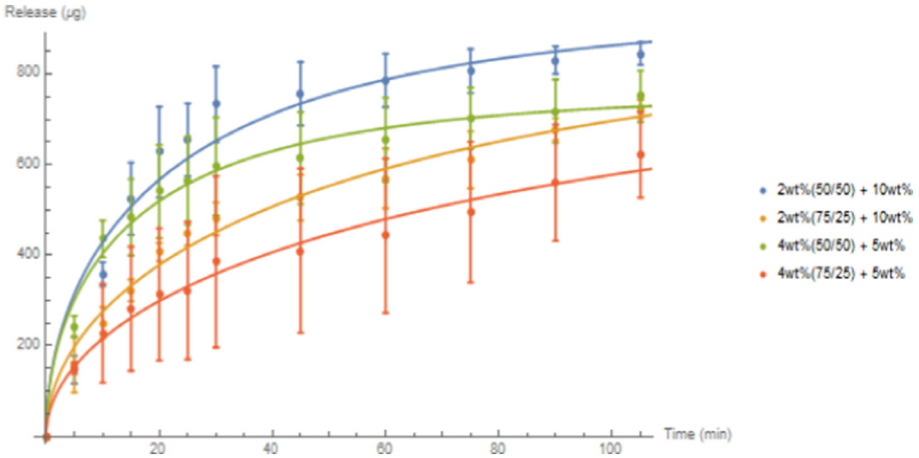


Fig. 6. Comparison of drug released from PLGA particles with analytical model predictions.

performed a similar analysis of previously published data [45], which gave a graph showing the release profiles of progesterone from 50 μm diameter 50 μm high microsylinders of 50:50 PLGA. Fitting those results gave a diffusion coefficient of 2.1×10^{-17} m^2 s^{-1}, which is in reasonable agreement with our results from much smaller particles.

4. Conclusions

In this work, the release kinetics of hydrophobic drug (progesterone) from drug/PLGA particles with different copolymer ratios and polymer/drug ratios were measured *in vitro*. It was found that the copolymer composition and polymer/drug ratio influence drug release kinetics at 37 °C. Overall, drug release rate was increased with increasing polymer concentration and with an increase from the PLGA with lower PLA: PGA copolymer ratio. In addition, this study also suggests that hydrophobic drugs can be successfully encapsulated into PLGA particles using electrospraying and the dissolution property of hydrophobic progesterone had been significantly improved after electrospray which can result in higher bioavailability. In conclusion, the drug release can be controlled significantly by varying the polymer concentration and copolymer composition for various applications.

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Author contributions

Y. Zhang, T. Shams – actual lab researchers.

A. H. Harker – Drug delivery calculations, graphs and related statistics.

M. Parhizkar and M. Edirisinghe – research supervisors who coordinated study and this paper.

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